# Vitamin Synthesis by the Symbionts in the Fat Body of the Cockroach, *Periplaneta americana* (L.)

DANIEL LUDWIG AND MARGARET R. GALLAGHER
DEPARTMENT OF BIOLOGICAL SCIENCES, FORDHAM UNIVERSITY

**Abstract:** Determinations were made on the vitamin content of the fat bodies of normal and aposymbiotic cockroaches. Of the 10 vitamins studied (ascorbic, folic, nicotinic and pantathenic acids, biotin, cyanocabalamin, inositol, pyridoxine, riboflavin and thiamine), only 3 (ascorbic, folic and pantathenic acids) were present in considerably larger amounts in the normal fat body. Cultured symbionts were able to synthesize them. The lighter cuticular color, sluggishness and reduced reproductive ability of the aposymbiotic insect may be explained by the absence of these vitamins.

Blochmann (1888), working with the cockroach, *Blatta orientalis*, was probably the first to observe intracellular bacteroids in the fat body of an insect. Glaser (1920, 1930) isolated the organisms, successfully cultured them and classified them as bacteria belonging to the genus *Corynebacterium*. Trager (1952), Peklo (1953), Brooks and Richards (1955a, b and 1956) all agreed that the bacteroids are intracellular symbionts.

Wigglesworth (1929) suggested that the role of the symbionts may be the synthesis of vitamins. He thought that the intracellular microorganisms in the fat body of the tsetse fly, *Glossina*, may synthesize vitamins necessary for growth. Evidence to support this view was given by Fraenkel and Blewett (1943a and b), Blewett and Fraenkel (1944), Pant and Fraenkel (1950, 1954) and Keller (1950), when they showed that insects with intracellular microorganisms did not, and those without them did, require most of the B vitamins in their diet. In addition to the B vitamins, there is evidence that the symbionts might be responsible for the synthesis of ascorbic acid. Filosa (1955) and Cordero (1956) demonstrated that homogenates of the cockroach, *Periplaneta americana*, can synthesize ascorbic acid using most of the D-sugars as substrates. Lisa (1958) observed that homogenates of the cockroach, *Leucophaea maderae*, synthesized ascorbic acid from D-mannose, and Pierre (1962), that the symbionts present in the fat body of this insect are responsible for this synthesis. Noland, Lilly and Baumann (1949) reported that the symbionts in the fat body of the cockroach, *Blatella germanica*, are largely responsible for the production of folic acid.

The present investigations, which consist of a comparison of the vitamin content of fat bodies of normal and aposymbiotic insects, were undertaken to determine whether vitamins are synthesized by the symbionts of the cockroach, *P. americana*.

Table 1. Methods used for the quantitative determination of vitamins in the fat bodies of normal and aposymbiotic cockroaches.

| Vitamin | Methods of assay |
|---|---|
| Ascorbic acid | Spectrophotometric method of Roe and Kuether (1942, 1943) with modifications of Lowry, Lopez and Bessey (1945) and by Mills and Roe (1947). |
| Biotin | Microbiological method of Pennington, Snell and Williams (1940), modified by the use of *Lactobacillus arabinosus* as given by Strohecker and Henning (1965).<br>Paper chromatographic method of Radhakrishnamurthy and Sarma (1953). |
| Cyanocobalamin | Microbiological method using *Lactobacillus leichmanii* ATCC 7830, outlined by Strohecker and Henning (1965). |
| Folic acid | Microbiological method of Capps, Hobbs and Fox (1948). |
| Inositol | Microbiological method of Stokes, Larsen, Woodward and Foster (1943).<br>Paper chromatographic method of Hough, Jones and Wadman (1948). |
| Nicotinic acid | Microbiological method of Snell and Wright (1941).<br>Paper chromatographic method of Kodicek and Reddi (1951). |
| Pantothenic acid | Microbiological method of Pennington, Snell and Williams (1940). |
| Pyridoxine | Microbiological method of Stokes, Larsen, Woodward and Foster (1943).<br>Paper chromatographic method of Snyder and Wender (1953). |
| Riboflavin | Microbiological method of Snell and Strong (1939).<br>Chemical method of Scott, Hill, Norris and Hensen (1946). |
| Thiamine | Microbiological method of Sarett and Cheldelin (1944).<br>Chemical method of Hennessy and Cerecedo (1939). |

## MATERIALS AND METHODS

The technique employed for rendering the cockroaches aposymbiotic was that of Brooks and Richards (1955a), except that they used a 0.1% antibiotic diet and the insects became aposymbiotic in the second generation; whereas in the present experiments, a 10% antibiotic diet was fed and they became aposymbiotic 120 days from the beginning of treatment. The diet consisted of 80% Gaines' dog pellets, 5% Brewer's yeast, 5% dextrose and 10% of a mixture of aureomycin and terramycin in a 1:1 ratio. The dog pellets were powdered and then mixed with the other ingredients. This food preparation was changed every 5 days to insure the freshness of the antibiotics. Controls were maintained on a diet of Gaines' dog pellets and water. Sub-groups of insects were cultured on diets which were deficient in the specific vitamin to be tested. Histological sections of the fat body were prepared at the end of 60, 90, 100 and 120 days to determine aposymbiosis.

Cultures of symbionts were obtained from the fat body according to the techniques of Begg and Sang (1950) and of Pant, Nayar and Gupta (1957). These cultures were maintained in lactose broth at 30° C.

Table 2. Amount of different vitamins found in the normal and aposymbiotic fat bodies of the cockroach. Values are given as amount/gram of fat body. Each is an average of 10 determinations.

| Vitamin | Normal | | | Aposymbiotic | | |
|---|---|---|---|---|---|---|
| | Micro-biological method | Chemical method | Chromato-graphic method | Micro-biological method | Chemical method | Chromato-grraphic method |
| Ascorbic acid | | 0.2 mg. | | | 0.03 mg. | |
| Biotin | 48.0 mμg. | | 42.0 mμg. | 45.0 mμg. | | 39.0 mμg. |
| Cyanocobalamin | 28.0 mμg. | | | 26.0 mμg. | | |
| Folic acid | 62.0 μg. | | | 9.6 μg. | | |
| Inositol | 126.8 μg. | | 120.0 μg. | 159.0 μg. | | 131.0 μg. |
| Niacin | 408.0 μg. | | 305.0 μg. | 528.0 μg. | | 420.0 μg. |
| Pantothenic acid | 74.0 μg. | | | 0.0 μg. | | |
| Pyridoxine | 67.6 mg. | | 61.0 mg. | 61.0 mg. | | 62.0 mg. |
| Riboflavin | 70.0 μg. | 70.0 μg. | | 68.0 μg. | 68.0 μg. | |
| Thiamine | 66.0 μg. | 71.0 μg. | | 62.0 μg. | 69.0 μg. | |

All analytical procedures were carried out on homogenates of fat bodies from normal and aposymbiotic nymphs. Five per cent homogenates were made in 0.2 molar phosphate buffer at a pH of 6.8, except for the determinations of riboflavin, nicotinic acid and thiamine, in which cases the fat bodies were homogenized in sterile distilled water. The various methods used to assay each vitamin are given in Table 1. Details of each are given by Gallagher (1962), and descriptions of the various methods for vitamin assays by Strohecker and Henning (1965).

## OBSERVATIONS

Organisms fed an antibiotic diet did not become completely aposymbiotic until 120 days of treatment. One manifestation of aposymbiosis was a change in the color of the cuticle from mahogany to a light tan. This change began approximately 80 days after the insect was placed on antibiotics. They also appeared less active, demonstrated a slower response on exposure to light and less speed in avoiding capture as compared to normal insects. They were also of smaller size and molted less frequently than normal insects.

The results of the vitamin assays are summarized in Table 2. The table shows that in all cases there is a close agreement in the results obtained by different methods for each of the vitamins. Of the 10 vitamins assayed, only 3 were present in smaller amounts in the fat bodies of the aposymbiotic than in those of the normal insect. They are ascorbic, folic and pantothenic acids. It appears that these vitamins are synthesized by the symbionts. Additional experiments, using cultures of isolated symbionts, verified this conclusion.

## DISCUSSION

The fading of the cuticular color in the aposymbiotic insect may be associated with the absence of ascorbic acid. In the normal insect, melanin is formed from the oxidation of tyrosine by tyrosinases. Ascorbic and pantothenic acids are activators of tyrosinase (Levine, Dann and Marples, 1943). In vertebrates, defective tyrosine metabolism can be corrected by the administration of either folic or ascorbic acids (Rodney, Swendseed and Swanson, 1947). If the reactions involving ascorbic, folic and pantothenic acids are similar in insects to those in vertebrates, a deficiency of any or all of them could produce a fading of the cuticular color. The present experiments demonstrate that they are all produced by the symbionts cultured from the fat body and are absent from the fat body of the aposymbiotic cockroaches. Henry (1962) reported that another deficiency of the aposymbiotic cockroach, *Blatella germanica*, is the inability to synthesize certain amino acids, including tyrosine, from glucose. Thus in insects without symbionts, the substrate from which melanins are formed is also lacking.

The decrease in reproductive capacity noted in the aposymbiotic insect may be caused by a deficiency of folic acid. Berger (1944) gave the first cytological evidence of the necessity of this vitamin for cell division when he showed that sulfanilamide, a folic acid antagonist, caused metaphase arrest in onion roots. Hindmarsh (1949) found that this inhibition of mitosis could be reversed with p-aminobenzoic acid, a precursor of folic acid. Goldsmith and Grank (1952) induced sterility in the vinegar fly, *Drosophila melanogaster*, by inhibiting mitosis in the germ cells with aminopterin, a folic acid antagonist. Mitlin, Butt and Shortino (1957) prevented oviposition in the house fly, *Musca domestica*, by feeding aminopterin. A microscopic examination of the ovaries showed inhibited ovarian growth and the eggs contained much less yolk than those of normal flies. Gersdorff and Mitlin (1954) showed that the addition of folic acid to the rearing medium reversed the antagonism of aminopterin in house fly larvae.

### Literature Cited

Begg, M. and J. H. Sang. 1950. A method of collecting and sterilizing large numbers of *Drosophila* eggs. Science, **112**: 11–12.

Berger, C. A. 1944. Experimental studies on the cytology of *Allium*. Torreya, **44**: 41.

Blewett, M. and G. Fraenkel. 1944. Intracellular symbionts and vitamin requirements in insects. Proc. Roy. Soc. London, **132**: 212–222.

Blochmann, F. 1888. Über des regelmässige Vorkommen von bakterienähnlichen Gebilden in den Geweben und Eieren verscheidener Insekten. Zeitschr. f. Biol., **24**: 204–224.

Brooks, M. A., and A. G. Richards. 1955a. Intracellular symbiosis in cockroaches. I. Production of aposymbiotic cockroaches. Biol. Bull., **109**: 22–39.

———. 1955b. Intracellular symbiosis in cockroaches. II. Mitotic division of the mycetocytes. Science, **122**: 242.

———. 1956. Intracellular symbiosis in cockroaches. III. Reinfection of aposymbiotic cockroaches with symbionts. J. Exp. Zool., **132**: 447–465.

CAPPS, B. F., N. L. HOBBS AND S. H. FOX. 1948. A dehydrated experimental medium for the microbiological assay of folic acid. J. Bact., **55**: 869–870.

CORDERO, S. M. 1956. The synthesis of ascorbic acid in the cockroach, *Periplaneta americana* (Linnaeus). M. S. Dissertation, Fordham University, New York.

FILOSA, M. 1955. A synthesis of ascorbic acid by nymphs of the cockroach, *Periplaneta americana* (Linnaeus). M. S. Dissertation, Fordham University, New York.

FRAENKEL, G. AND M. BLEWETT. 1943a. Vitamins of the B group required by insects. Nature, **151**: 703.

———. 1943b. Intracellular symbionts of insects as a source of vitamins. Nature, **152**: 506.

GALLAGHER, M. R. 1962. Vitamin synthesis by the symbionts in the fat body of the cockroach *Periplaneta americana*. Ph.D. Dissertation, Fordham University, New York.

GERSDORFF, W. A. AND N. MITLIN. 1954. The relative toxicity to house flies of the methyl and ethyl analogs of allethin. J. Econ. Ent., **46**: 945–948.

GLASER, R. W. 1920. Biological studies on intracellular bacteria. Biol. Bull., **39**: 133–144.

———. 1930. On the isolation, cultivation and classification of the so-called intracellular "symbiont" or "rickettsia" of *Periplaneta americana*. J. Exp. Med., **51**: 59–81.

GOLDSMITH, R. D. AND I. GRANK. 1952. Sterility in the female fruit fly, *Drosophila melanogaster*, produced by the feeding of a folic acid antagonist. Amer. J. Physiol., **171**: 726–729.

HENNESSY, D. AND L. R. CERECEDO. 1939. The determination of free and phosphorylated thiamine by a modified thiochrome assay. J. Amer. Chem. Soc., **61**: 179–183.

HENRY, S. M. 1962. The significance of microorganisms in the nutrition of insects. Trans. N. Y. Acad. Sci., Ser. II, **24**: 676–683.

HINDMARSH, M. M. 1949. Effects of sulphanilamide and p-aminobenzoic acid on mitosis. Nature, **163**: 610.

HOUGH, L., J. K. N. JONES AND W. H. WADMAN. 1948. Applications of paper chromatography to the separation of the sugars and their derivatives on a column of powered cellulose. Nature, **162**: 448–449.

KELLER, H. 1950. Die Kultur der intrazellularen Symbioten von *Periplaneta orientalis*. Zeitschr. f. Naturforsch., **56**: 269–273.

KODICEK, E. AND K. K. REDDI. 1951. Paper chromatographic determination of nicotinic acid and its derivatives. Nature, **168**: 475–477.

LEVINE, S. Z., M. DANN AND E. MARPLES. 1943. A defect in the metabolism of tyrosine and phenylalanine in premature infants. III. Demonstration of the irreversible conversion of phenylalanine to tyrosine in human organisms. J. Clin. Invest., **22**: 551–552.

LISA, J. D. 1958. Enzymes of the fat body of the cockroach, *Leucophaea maderae* (Fabricius). Ph.D. Dissertation, Fordham University, New York.

LOWRY, O. D., H. A. LOPEZ AND O. A. BESSEY. 1945. The determination of ascorbic acid in small amounts in blood serum. J. Biol. Chem., **160**: 609–612.

MILLS, M. B. AND J. H. ROE. 1947. A critical study of proposed modifications of the Roe and Kuether method for the determination of ascorbic acid, with further contributions to the chemistry of this procedure. J. Biol. Chem., **170**: 159–163.

MITLIN, N., C. BUTT AND J. SHORTINO. 1957. Effect of mitotic poisons on house fly oviposition. Physiol. Zool., **30**: 133–136.

NOLAND, J. L., J. H. LILLY AND C. A. BAUMANN. 1949. Vitamin requirements of the cockroach *Blatella germanica* (L.). Ann. Ent. Soc. Amer., **42**: 154–164.

PANT, N. C. AND G. FRAENKEL. 1950. The function of symbiotic yeasts of two species of insects, *Lasioderma serricorne* and *Stegobium* (*Sitodrepa*) *paniceum*. Science, **112**: 498.

———. 1954. On the functions of intracellular symbionts of *Oryzaephilus surinamensis*. J. Zool. Soc. India, **6**: 191–192.

———, J. K. Nayar and P. Gupta. 1957. On the isolation and cultivation of the intracellur symbionts of *Oryzaephilus surinamensis*. Experimentia, **13**: 241.

Peklo, J. 1953. Microorganisms or mitochondria? Science, **118**: 202.

Pennington, D., E. E. Snell and R. J. Williams. 1940. Effect of diet on pantothenic acid content of chick tissues. J. Biol. Chem., **135**: 212–222.

Pierre, L. L. 1962. Synthesis of ascorbic acid by the normal fat body of the cockroach, *Leucophaea maderae* (F.), and by its symbionts. Nature, **193**: 904–905.

Radhakrishnamurthy, R. and P. S. Sarma. 1953. Symposium on chromatography. **11**: 10–46.

Rodney, G., M. E. Swendseed and A. L. Swanson. 1947. Tyrosine oxidation by livers in rats with sulfasuxidine-induced pteroylglutamine acid deficiency. J. Biol. Chem., **168**: 395–396.

Roe, J. H. and C. A. Kuether. 1942. A color reaction for dehydroascorbic acid useful in the determination of vitamin C. Science, **95**: 77–84.

———. 1943. The determination of ascorbic acid in whole blood and urine through the 2, 4-dinitrophenylhydrazine derivative of dehydroascorbic acid. J. Biol. Chem., **150**: 609–613.

Sarett, H. P. and V. H. Cheldelin. 1944. Use of *Lactobacillus fermentum* 36 for thiamine assay. J. Biol. Chem., **155**: 153–160.

Scott, M. L., F. W. Hill, L. C. Norris and G. F. Hensen. 1946. Clinical determination of riboflavin. J. Biol. Chem., **165**: 65–71.

Snell, E. E. and G. Strong. 1939. Microbiological assay of riboflavin. Indust. Engin. Anal., **11**: 346–348.

——— and L. D. Wright. 1941. A microbiological method for the determination of nicotinic acid. J. Biol. Chem., **139**: 675–686.

Snyder, J. W. and S. H. Wender. 1953. Separation and determination of pyridoxine, pyridoxal and pyridoxamine by paper chromatography. Arch. Biochem. Biophys., **46**: 465–469.

Stokes, E., A. Larsen, H. G. Woodward and R. Foster. 1943. Neurospora assay for pyridoxine. J. Biol. Chem., **150**: 17–24.

Strohecker, R. and H. M. Henning. 1965. Vitamin Assay. The Chemical Rubber Co., Cleveland.

Trager, W. 1952. Mitochondria or microorganisms? Science, **116**: 332.

Wigglesworth, V. B. 1929. Digestion in the tse-tse fly. Parasit., **21**: 316.

Received for publication May 17, 1966